Supporting Information

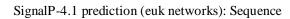
A basic protein, N25, from a mollusk modifies calcium carbonate morphology and shell biomineralization

Dong Yang¹, Yi Yan¹, Xue Yang¹, Jun Liu¹, Guilan Zheng¹, Liping Xie^{1*}, Rongqing Zhang^{1,2*}

- 1, Institute of Marine Biotechnology, Collaborative Innovation Center of Deep Sea Biology, School of Life Sciences, Tsinghua University, Beijing 100084, China
- 2, Department of Biotechnology and Biomedicine, Yangtze Delta Region Institute of Tsinghua University, Jiaxing, Zhejiang Province 314006, China

Running title: Functions of N25 on CaCO₃ morphology

* Correspondence should be addressed: Rongqing Zhang: rqzhang@mail.tsinghua.edu.cn; Liping Xie: lpxie@mail.tsinghua.edu.cn; Tel.: +86 010 62776230; fax: +86 010 62772899.



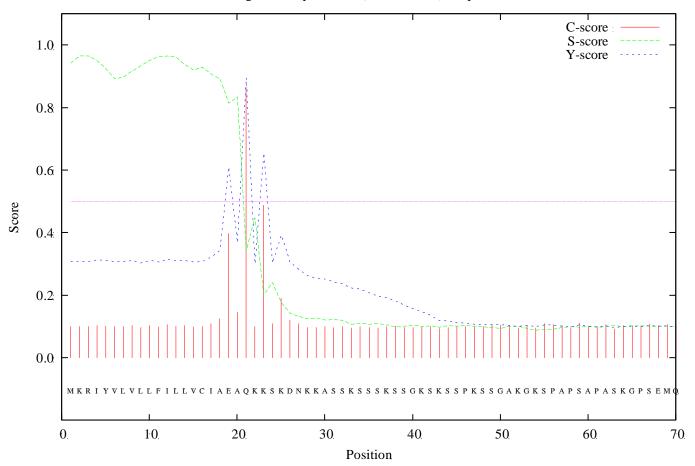


Figure S1. The signal peptide prediction with the cleavage site at EA-QKK.

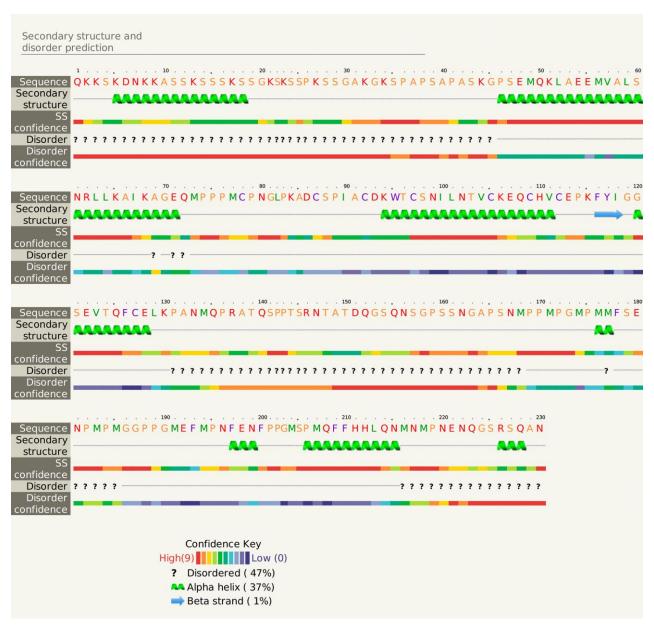


Figure S2. The secondary structure prediction from the primary sequence. The graphic symbols were illustrated as shown in picture. (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index)

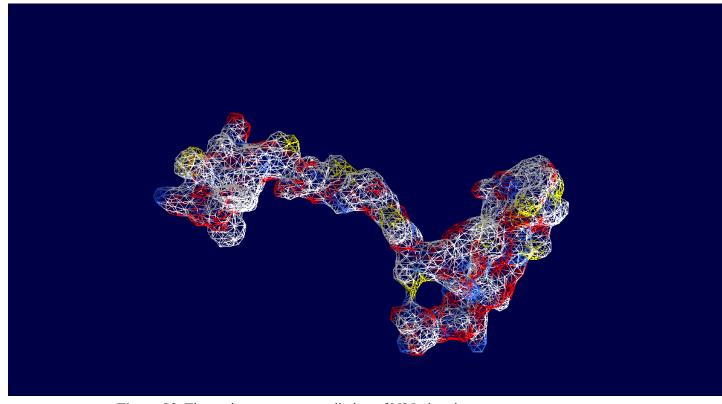


Figure S3. The tertiary structure prediction of N25 via primary sequence. (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index)

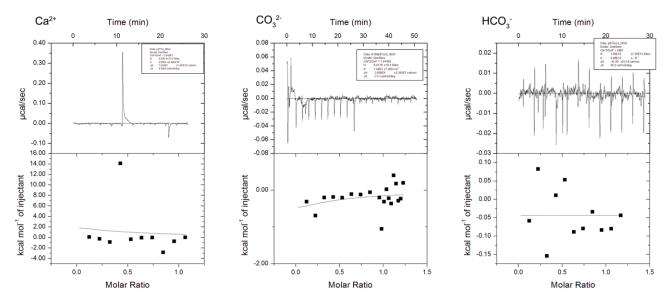


Figure S4. Isothermal Titration Calorimetry (ITC) results of N25 titrated with CaCl₂, Na₂CO₃ or NaHCO₃ respectively. The concentration of N25 was about 1.0 mg/mL, the concentrations of Ca²⁺, CO₃²⁻ and HCO₃⁻ were about 50 μ M.

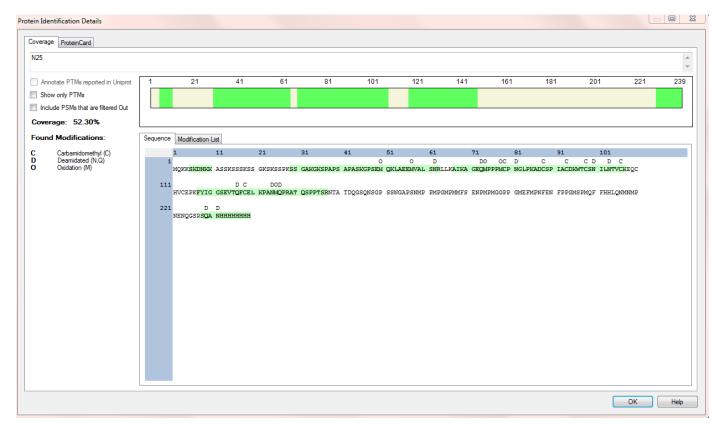


Figure S5. N25 protein identification by mass spectrometry and sequence coverage of the band in Figure 1A.

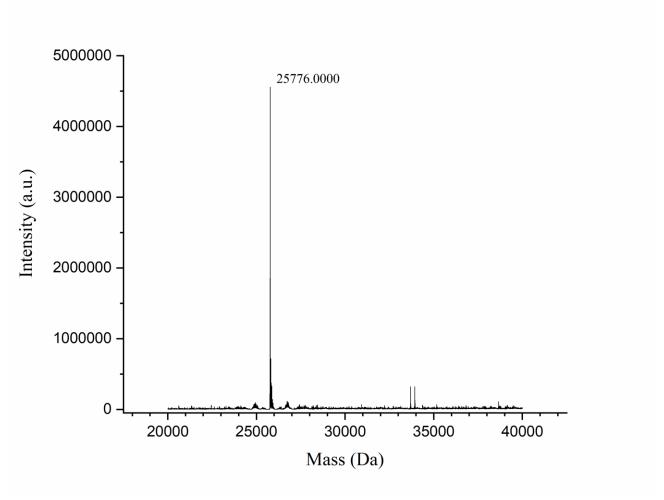


Figure S6. Analysis of N25 protein molecule weight by QTOF-MS.

Table S1. The post-translational modifications analysis of N25 by MS

-10lgP	Coverage	Area Sample 1	#Peptides	#Unique	#Spec Sample 1	PTM	Avg. Mass
461.66	95%	1.98E+11	237	237	5033	Oxidation (M); Carbamidomethylation; Acetylation (K); Acetylation (N-term); Amidation; 206 more	25652